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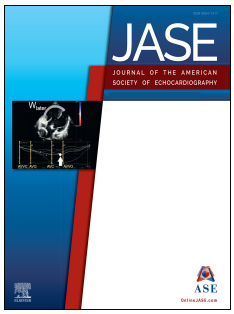

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Exercise-induced cardiac fatigue after a 45-minute bout of high-intensity running exercise is not altered under hypoxia

Brief title: Exercise-induced cardiac fatigue under hypoxia

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ABSTRACT

Background. Acute exercise promotes transient exercise-induced cardiac fatigue (EICF), which affects the right ventricle (RV) and to a lesser extent the left ventricle (LV). Hypoxic exposure induces an additional increase in RV afterload. Therefore, exercise in hypoxia may differently affect both ventricles.

Aim. Investigate the acute effects of a bout of high-intensity exercise under hypoxia *versus* normoxia in healthy individuals on right- and left-sided cardiac function and mechanics.

Methods. 21 healthy individuals (22.2±3.0 years, fourteen men) performed a 45-minute high-intensity running exercise, under hypoxia (fraction of inspired oxygen [FiO_2] 14.5%) and normoxia (FiO_2 20.9%) in a randomized order. Pre- and post-exercise echocardiography, at rest and during low-to-moderate intensity recumbent exercise ('stress'), was performed to assess RV and LV cardiac function and mechanics. RV structure, function and mechanics were assessed using conventional 2D, Doppler, tissue Doppler, speckle tracking echocardiography and novel strain-area loops.

Results. Indices for RV systolic function (RVFAC, TAPSE, RVS', RV free wall strain) as well as LV function (LV ejection fraction, LV global longitudinal strain)) significantly reduced after high-intensity running exercise ($p<0.01$). These exercise-induced changes were more pronounced when echocardiography was examined during stress compared to baseline. These responses in RV or LV were not altered under hypoxia ($p>0.05$).

Conclusion. There was no impact of hypoxia on the magnitude of EICF in the RV and LV after a bout of 45-minute high-intensity exercise. This finding suggests that any potential increase in loading conditions does not automatically exacerbate EICF in this setting.

Keywords: athlete's heart; exercise-induced cardiac fatigue; hypoxia; echocardiography; speckle tracking echocardiography

INTRODUCTION

It is well established that exercise is associated with potent cardioprotective effects[1-3], however, acute exercise can lead to a paradoxical short-term increase in cardiac events.[4-6] One potential explanation is that exercise performed under demanding conditions (i.e. exercise at high-intensity and/or during prolonged duration) may lead to an acute reduction in cardiac function.[7-13] This transient decline in cardiac function after strenuous exercise is typically referred to as exercise-induced cardiac fatigue (EICF). EICF may affect both left (LV) and right ventricles (RV), with possibly a larger impact on the RV due to the disproportionately higher wall stress experienced by the RV relative to the LV during exercise.[11, 14, 15]

Previous studies have demonstrated that hypoxia increases the demands on the cardiovascular system.[16] Specifically, acute exposure to hypoxia induces a decrease in systemic vascular resistance at rest, which may contribute to a decrease in LV afterload.[17, 18] In contrast, hypoxia leads to a resting increase in pulmonary artery resistance, and subsequently to an increase in pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP).[19] Exercise in normoxic conditions results in additional load challenges and an increased PAP secondary to the mismatch of elevated stroke volume to inadequate pulmonary vascular distension.[20] This is exacerbated when exercising in hypoxic conditions, leading to an even greater PAP and RV wall stress and potentially further increasing the risk of RV EICF.[19-23]

To non-invasively examine right heart haemodynamics, studies have examined conventional and Doppler based echocardiographic indices at rest and during exercise.[24-26] Recently, the strain-area loop has been introduced assessing simultaneous structure and strain across the cardiac cycle.[5] Previously, we found that RV loop characteristics relate to PVR in patients with pulmonary arterial hypertension (PAH) whilst also demonstrating value in the assessment of EICF.[27, 28] Therefore, these non-invasive characteristics may provide additional insight in understanding exercise-induced changes in hypoxia.

In view of this, the aim of this study, was to investigate the acute effects of a bout of high-intensity exercise under hypoxia *versus* normoxia in healthy individuals on right- and left-sided cardiac function and mechanics (i.e. longitudinal strain and strain-area loops). Based on the presumed higher workload of the RV during hypoxic *versus* normoxic exercise, we hypothesize that exercise under hypoxia exaggerates RV to a greater extent than LV compared to exercise under normoxia. To investigate EICF, we examined pre- and post-exercise echocardiography at rest, but also during a standardized low-to-moderate-intensity recumbent exercise challenge ('stress'). As the post-exercise recovery period is associated with persistent sympathoexcitation and peripheral vasodilation[17, 18], evaluation of EICF could be confounded when evaluated solely at rest. Therefore, evaluation during stress echocardiography may better reflect cardiac function *during* exercise and offsets the key limitation of (para)sympathetic imbalance associated with echocardiographic assessment in recovery.[14]

METHODS

Study population

Twenty-one participants (22.2±0.6 years, fourteen men, 24.0±0.6 kg/m^2, VO_2max/kg/min 52.4±1.8 mL/min/kg) completed the study. Baseline characteristics are shown in **Table 1**. Participants were eligible to take part in this study if they were able to run on a treadmill and if they trained <2 hours a week at moderate-to-high-intensity for the last six months. Exclusion criteria were a body mass index (BMI) <18 or >30 kg/m^2, active smoker, any possibility of pregnancy, personal history of cardiovascular disease, positive family history of cardiovascular death (<55y), exercise-limiting respiratory disease, physical (i.e. musculoskeletal) complaints making completion of a bout of high-intensity running exercise impossible, abnormal resting 12-lead electrocardiogram (ECG) and abnormalities observed on resting transthoracic echocardiography. The procedures were in accordance with institutional guidelines and conformed to the declaration of Helsinki. The study was

approved by the Ethics Research Committee of Liverpool John Moores University (18/SPS/065). Participants gave full written and verbal informed consent before participation.

Study design

In this randomized crossover trial, participants attended the laboratory on three separate occasions (**Figure 1**). During the first visit, a medical screening was performed to determine eligibility of the potential participants. After signing informed consent, baseline measurements were performed. Visits two and three included performance of a bout of 45-minute high-intensity running exercise under normobaric hypoxia or normoxia, which were performed in a randomized order. Participants were blinded for the order of test days and abstained from exercise for a minimum of 48 hours, and from alcohol and caffeine consumption 24 hours before the test days.

Baseline measurements. Participants were examined for height (SECA stadiometer, SECA GmbH, Germany), weight (SECA scale, SECA GmbH, Germany), oxygen saturation (SpO_2, pulse oximetry; Ana Pulse 100, Ana Wiz Ltd., UK), 12-lead ECG (Cardiovit MS-2010, Schiller, Switzerland) and maximal oxygen consumption (VO_2max). Resting heart rate (HR, Polar, Kempele, Finland) and resting blood pressure (BP, Dinamap V100, GE Medical, Norway) were determined at the end of ten minutes of quiet rest in a supine position. A standardized maximal cardiopulmonary exercise test (CPET, Oxycon pro, CareFusion, VS) for VO_2max assessment was conducted on a motorized treadmill (HP Cosmos, Nussdorf, Germany) after a 10-min warm-up and familiarization. VO_2max was defined as the highest value of a 30-s average[29], and attainment was verified according to previous recommended criteria.[30]

Test days. **Figure 1** outlines the details of a single test day. One of the test days was performed at normoxia (sea level, equivalent to fraction of inspired oxygen [FiO_2] 20.9%) and the other at normobaric hypoxia (FiO_2 14.5%; equivalent to a simulated altitude of 3,000m), separated by at least 48 hours of rest. Participants were subjected to 30 minutes of acclimation in a seated position followed by 45-minute of high-intensity (85% of maximum achieved HR during CPET) endurance

running exercise on a motorized treadmill (HP Cosmos, Nussdorf, Germany) and 60 minutes of recovery in seated position. HR was measured continuously throughout (Polar, Kempele, Finland), and rate of perceived exertion (RPE) was monitored during the 45-minutes high-intensity running exercise.[31]

In total four echocardiographic assessments were performed per test day. After acclimation and prior to the 45-minute exercise, echocardiography was performed under resting conditions ('rest') and during recumbent cycling to elevate heart rate to directly assess cardiac function during exercise ('stress', target HR 110-120 bpm). The 'stress' echocardiogram was repeated directly after the 45-minute exercise, to prevent sympathetic withdrawal (i.e. a drop in BP and HR).[32] Finally, images were obtained at the end of the 60 minutes of recovery in a resting state. During every echocardiography assessment, BP measurements were performed. Measurements were performed at the same time on both days to control for diurnal variation. Fluid intake was controlled by providing the same amount of water to participants during both testing days.

Environmental chamber and safety. All exercise tests were conducted in an environmental chamber (TISS, Alton, UK; Sportingedge, Basingstoke, UK). Normobaric hypoxia was achieved by a nitrogen dilution technique. Ambient temperature, carbon dioxide (CO_2) and oxygen (O_2) levels were controlled in all sessions (20°C; FiO_2 14.5%; CO_2 0.03%), whilst a Servomex gas analysis system (Servomex MiniMP 5200, Servomex Group Ltd., UK) was used inside the chamber to provide the researcher continuous information regarding O_2 and CO_2 levels. Acute mountain sickness symptoms (AMS, measured by Lake Louise Score[33] (LLS)) were monitored during testing and training sessions every 20 minutes. The subject was removed from the environmental chamber if oxygen saturation levels dropped below 80% or severe AMS was suspected (LLS≥6).

Echocardiographic measurements

Rest and stress echocardiography were performed in the left lateral decubitus position on a supine cycle ergometer (Lode B.V.; Groningen, The Netherlands) by one highly experienced sonographer (DO) using a Vivid E95 ultrasound machine (GE Medical, Horton, Norway), equipped with a 1.5-4.5

MHz transducer. Images were stored in raw digital imaging and communication in medicine (DICOM) format and were exported to an offline workstation (EchoPac, version 203, GE Medical, Horton, Norway). Data-analysis, from three stored cycles, was performed by a single observer with experience in echocardiography (GK) using commercially available software (EchoPac, version 203, GE Medical, Horton, Norway). The observer was blinded for the timing (pre *vs*. post) and condition (normoxia *vs*. hypoxia) under which echocardiography was performed. For stress echocardiography, low-to-moderate-intensity (target HR 110-120 bpm) exercise consisted of recumbent cycling at a cadence of ~60 revolutions per minute with watts manually adjusted to stabilise at target HR.

Conventional measurements. Cardiac structural and functional measurements were made according to the current guidelines for cardiac chamber quantification.[34] Regarding the right heart, we examined the following structural and functional indices: basal and mid-cavity end-diastolic diameters, RV end-diastolic area (RVEDA), RV end-systolic area (RVESA), RV outflow tract (RVOT) diameter at the proximal level in the parasternal long-axis (PLAX) and parasternal short-axis (PSAX) view, right atrial (RA) area, RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE), tissue Doppler imaging (TDI) of the tricuspid annulus (RV 's, e', a') and pulmonary artery Doppler acceleration time (PAT). Tricuspid regurgitation velocity was not obtainable in the major part of the participants and therefore was unable to be utilized in this study.

Regarding the left heart, the following structural and functional indices were determined: LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LA diameter, LA volume, modified Simpson's left ventricular ejection fraction (LVEF), tissue Doppler imaging (TDI) of the mitral annulus (LV ', e' and a') and trans-mitral Doppler (E, A and E/A ratio). Doppler A and RV and LV TDI a' were not measurable on account of e'/a' and E/A fusion during stress echocardiography at higher heart rates.

Mechanics. Images were acquired and optimized for STE. This involved maintaining frame rates between 40 and 90 frames s^{-1}, depth to ensure adequate imaging of the chamber of interest and

compression and reject to ensure endocardial delineation. The RV focused and the apical two-chamber, four-chamber and long-axis view were utilized for the RV and LV global longitudinal strain, respectively. Pulmonary and aortic valve closure times were determined from the respective pulsed wave Doppler signals. For both the RV and LV views the myocardium was manually traced to include the septum and adjusted so that the region of interest (ROI) incorporated all of the wall thickness while avoiding the pericardium.[35, 36] The region of interest was divided into six myocardial segments, providing segmental strain curves and a longitudinal strain curve as an average of all six segments for the LV views and as an average of the 3 segments of the RV free wall. LV global longitudinal strain (LVGLS) was obtained by averaging the single strain measurements of the three separate apical LV views. If inappropriate tracking of segments was observed visually or detected by the system, retracing was performed until all segments were considered acceptable.

RV strain-area loops. The longitudinal strain-area relationship (detailed methods of derivation see, Supplemental 1, Oxborough *et al.*[5] and Hulshof *et al.*[37]) was assessed using the following parameters (**Figure 2**): (I) the linear strain-area slope (Sslope) and early strain-area slope during first 5% of volume ejection in systole (ESslope); (II) end-systolic peak longitudinal strain (peak strain); (III) the early linear strain-area slope during first 5% (EDslope) and late linear strain-area slope (LDslope) during last 5% of volume increase in diastole; and (IV) diastolic uncoupling (i.e. difference in strain between systole and diastole at any given area), divided into uncoupling during early (Uncoupling ED) and late diastole (Uncoupling LD).[5, 28] Based on previous work from our laboratory, we found that PAH patients with higher PVR have a lower Sslope and a decreased Uncoupling LD. Therefore, these may serve as markers of an increased PVR and consequently PAP.[28]

In order to obtain intra-observer variability, strain-area loops were re-analysed in 20 randomly selected echocardiograms (n=10 rest, n=10 stress). For all strain-area loop characteristics intra-class correlation coefficient (ICC) and Bland-Altman limits of agreement (LOA) analysis were performed.[38]

Statistical analysis

Statistical analysis was performed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, VS). All parameters were visually inspected for normality and tested with Shapiro-Wilk normality tests. Continuous variables were reported as mean ± standard error of the mean (SEM) and categorical variables were presented as proportions. Linear mixed models analysis for repeated measurements were performed to test the acute effects of a bout of 45-minutes high-intensity exercise on cardiac function and mechanics (Exercise), and whether this effect was influenced when echocardiography was performed at rest or during stress (Exercise*Stress). Furthermore, linear mixed models were used to test the effect of hypoxia *versus* normoxia (Hypoxia) and the effect of rest versus stress echocardiography (Stress) on cardiac structure and function. To examine our primary objective, linear mixed models analysis was used to examine whether hypoxia impacted the effect of exercise on cardiac function (Exercise*Hypoxia), and how this was affected by testing condition rest *versus* stress (Exercise*Hypoxia*Stress). For all tests, we assumed statistical significance at $p<0.05$.

RESULTS

Both the right and left heart had normal geometry and all structural measurements were within normal ranges (**Table 2**). There were no abnormal 12-lead ECG findings.

Exercise characteristics. HR during exercise was matched between exercise under hypoxia and normoxia (172±1 bpm, 173±2 bpm respectively, p=0.23). Body mass loss (hypoxia -410±70g *vs*. normoxia -410±43g p=0.99) and water intake (hypoxia 373±60ml *vs*. normoxia 336±44ml, p=0.24) during exercise did not differ between testing sessions. Mean distance covered during exercise was significantly higher in normoxia (6,655±351m) compared to hypoxia (5,797±308m, p<0.001), whilst there was no significant difference in subjective ratings of perceived exertion (RPE normoxia 12.5±0.3, RPE hypoxia 13.3±0.3; p=0.07). SpO_2 during exercise was significantly lower in hypoxia (82±0.8) compared to normoxia (95±0.4).

232

233 **Right ventricular structure, function and mechanics**

234 All RV structural, functional and mechanicals indices pre- and post- 45-minute high-intensity running
235 exercise are displayed in **Table 2**. Indices of RV systolic function (RVFAC, TAPSE, RVS', RV free wall
236 strain (**Figure 3A**)) significantly reduced following 45-minute high-intensity exercise (Exercise:
237 $p<0.01$). The decline in indices of RV function and mechanics after exercise were not different
238 between rest and stress echocardiography, except for a more pronounced reduction in RV free wall
239 strain during stress (Exercise*Stress: $p=0.01$, **Table 2**, **Figure 3A**)). Related to the strain-area loop,
240 following 45-minute high-intensity exercise there was a reduction in RV longitudinal strain,
241 uncoupling and uncoupling LD (Exercise: $p<0.05$) without a rightward shift (RVEDA Exercise: $p>0.05$)
242 (**Table 2 Figure 4A,B**).

243 *Exercise under hypoxia.* Under hypoxia, PAT was significantly shorter, RA size significantly larger, late
244 diastolic uncoupling (Uncoupling LD) significantly lower, and a trend was found for a lower systolic
245 slope (Sslope) compared to normoxic conditions (Hypoxia: $p=0.04$, $p=0.04$, $p<0.001$, $p=0.07$,
246 respectively, **Table 2, Figure 4A,B**). Importantly, hypoxia did not alter the impact of exercise and/or
247 stress on indices of RV function (Hypoxia*Exercise and Exercise*Hypoxia*Stress: all $p>0.05$, **Table 2**).

248 *Intra-observer variability*. ICC and LOA for RV strain-area loop characteristics were as follows: RV free
249 wall strain ICC 0.95 (0.89-0.98), LOA 0.33 (-1.55, 2.21); Sslope ICC 0.91 (0.80-0.97), LOA -0.05 (-0.30,
250 0.20); ESslope ICC 0.60 (0.23-0.82), LOA 0.60 -0.17 (-1.20, 0.86); EDslope ICC 0.93 (0.84-0.97), LOA
251 0.19 (-0.37, 0.75); LDslope ICC 0.95 (0.87-0.98), LOA -0.30 (-0.93, 0.32); Uncoupling ICC 0.88 (0.73-
252 0.95), LOA -0.27 (-2.36, 1.81); Uncoupling_ED ICC 0.86 (0.68-0.94), LOA -0.31 (-2.63, 2.01);
253 Uncoupling_LD ICC 0.88 (0.72-0.95), LOA -0.20 (-2.25, 1.86).

254

255 **Left ventricular structure, function and mechanics**

All LV structural, functional and mechanicals indices pre- and post- 45-minute high-intensity running exercise are displayed in **Table 3**. With the exception of LVS' (Exercise: p=0.78), indices of LV systolic function (LVEF, LVGLS) significantly reduced following high-intensity exercise (Exercise: p<0.001). The reduction in LVEF and LVGLS was more pronounced in stress *versus* rest echocardiography (Exercise*Stress: both p<0.05, **Figure 3B**).

Exercise under hypoxia. Changes in LV indices in response to exercise, either examined at rest and/or during stress, were not different when performed under hypoxic conditions (Hypoxia*Exercise and Exercise*Hypoxia*Stress: p>0.05, **Table 3**). Blood pressure response patterns did not significantly differ between hypoxic and normoxic conditions (Hypoxia and Hypoxia*Exercise: all p>0.05, **Table 3**).

DISCUSSION

The aim of our study was to investigate the impact of a bout of high-intensity exercise under hypoxia *versus* normoxia on EICF on both ventricles. The main findings were 1) a bout of 45-minute high-intensity exercise induced a reduction in functional indices of right- and left-sided cardiac function and mechanics in healthy individuals, 2) the reduction in right- and left-sided cardiac function was more pronounced when echocardiography was performed during a standardized low-to-moderate-intensity recumbent exercise challenge and 3) there was no impact of hypoxia on exercise-induced reduction in right- or left-sided cardiac function and mechanics, either under rest or under stress. Taken together, these data indicate that EICF after short-term high-intensity exercise is not exaggerated under hypoxia, suggesting that an additional cardiac load (induced by hypoxia) on the RV does not necessarily relate to an exaggerated EICF in this setting.

High-intensity exercise-induced cardiac fatigue

A bout of 45-minute high-intensity running exercise induced a reduction of both RV and LV function indicative for EICF, which was mainly expressed during a low-to-moderate-intensity exercise challenge ('stress') compared to resting conditions. Earlier studies primarily investigated EICF after prolonged exercise (>180minutes)[4, 27], however, recent research has revealed a dose-response relationship between EICF and the duration and intensity of exercise.[14, 39] Our study adds the novel knowledge that EICF also occurs after relatively short periods of high-intensity exercise in both the RV and LV. Interestingly, in contrast to other short-term high-intensity EICF studies[10, 14, 39], we showed also marked reductions in LV function which may be due to the different type of exercise (running *vs.* cycling). An explanation for our ability to detect EICF after a relatively short duration of exercise may relate to the post-exercise assessment of cardiac function during 'stress', i.e. low-to-moderate-intensity exercise. Indeed, some of the indices for systolic function were primarily/only reduced when echocardiography was performed during the low-to-moderate-intensity exercise challenge. For example, a reduction in RVLS post-exercise was only apparent during the low-to-moderate-intensity exercise challenge (**Figure 4A**). We believe the echocardiography assessment under low-to-moderate-intensity exercise is more likely to detect EICF. The recovery phase post-exercise is associated with a change in autonomic tone and vasodilation, which may result in post-exercise tachycardia and hypotension, respectively. These (para)sympathetic imbalance factors likely influence cardiac function measurements such as strain, and therefore potentially mask the presence of EICF. Evaluation of cardiac function *during* the high-intensity exercise, therefore, is preferred. However, one should consider the practical aspects (e.g. echocardiography is impossible during running) and that reliable speckle tracking is extremely challenging with higher heart rates (i.e. 70% of maximum HR).[40] Low-to-moderate intensity cycling exercise at a semi-recumbent bike is both feasible and reliable, and allows to examine cardiac function during exercise. Utilising this approach, our data indicates that, with short durations of high-intensity exercise, EICF occurs when assessment of cardiac function is performed during an exercise challenge.

Impact of exercise under hypoxia

Under hypoxic conditions, less oxygen is bound to haemoglobin, and will, therefore, increase the demand on the cardiovascular system. In our population, this was reflected by a higher resting HR under hypoxia *versus* normoxia and the less distance covered under hypoxia *versus* normoxia during the exercise despite it being matched for relative intensity. More importantly, hypoxia has been shown to induce vasoconstriction of the pulmonary vasculature, leading to higher relative PVR resulting in a higher PAP, and consequently a higher RV wall stress. Elevated PAP has been previously demonstrated at conditions at 3000m altitude.[23] Although we were unable to directly measure PAP, we demonstrated shorter PAT and a larger RA size which indirectly supports the presence of an increase in PAP and, therefore potentially wall stress. Also, the strain-area loop showed less uncoupling in late diastole and a trend for a less steep systolic slope under hypoxia. In line with a previous study in PAH patients, these changes are associated with a higher PVR at rest.[28] Although we adopted a non-invasive approach and one should consider alternative explanations (i.e. related to the assessment), these findings support the presence of an elevated wall stress in our study under hypoxia. That aside, our hypothesis was rejected as the 45-minute high-intensity running exercise under hypoxia did not exaggerate RV EICF compared to exercise under normoxia. This suggests that changing cardiac workload does not necessarily change the magnitude of RV EICF and may not be the principle mechanism for RV EICF. One potential explanation for the lack of an impact of hypoxia on EICF may be that the exaggerated loading conditions under hypoxia were not sufficient enough at 3000m of simulated altitude, and/or the exposure time to the raised afterload of the RV was not long enough to contribute to the EICF magnitude. There are also indications that hypoxia itself may induce cardiac dysfunction due to sustained low oxygen availability, however, this seems mainly during prolonged exposure.[41]

Our hypothesis originated from the accepted phenomenon of disproportionately higher relative wall stress in the RV compared to the LV during exercise, but also based on observations suggesting a

larger magnitude of EICF in the RV compared to the LV.[11, 14, 15] For example, Stewart *et al.* examined the influence of high-intensity exercise on RV free wall and segmental LV strain EICF following 90 minutes cycling[10], and found that the reduction in strain was more profound in the RV than in the LV. In their study they demonstrated a relative reduction in RV strain of -17.5% compared to -9.8% in our study, which supports a dose-response relationship. Our study is the first to our knowledge to directly compare normoxic and hypoxic conditions on EICF, and demonstrated similar changes in both RV and the LV. Although mechanical changes in the RV and LV are independent of each other[27], and likely differ during exercise, our work suggests that (after)load dependency may be a less contributing factor to EICF as previously suggested. Alternatively, intrinsic myocardial factors such as β-adrenergic receptor desensitization[7, 42] and oxidative stress[43] may play a more substantial role. Our study, however, is unable to provide further insight into these other possible mechanisms.

It is also of interest that following the 45-minute high-intensity exercise, this study showed a lack of any RV dilation (no rightward shift strain-area loop, **Figure 4**) as previously demonstrated following prolonged exercise.[27] Previous studies have demonstrated a serial and parallel impact from ventricular interdependence on LV filling secondary to RV volume / pressure overload.[27, 44] This finding is consistent with other studies of high-intensity exercise of relative short durations rather than is seen in EICF studies of prolonged exercise highlighting a possible dose response related to both intensity and duration.[10, 14, 27] In the shorter duration exercise intervention studies, the reduction in LV size occurs irrespective of changes in RV size which provides additional support for an intrinsic mechanism independent to both the right and left side of the heart. Moreover, the decreased uncoupling in the strain-area loop (**Figure 4**), indicating less longitudinal contribution to area change, in combination with a lack of RV dilatation, supports that the reduction in peak longitudinal strain post-exercise (i.e. EICF) is more likely representative of intrinsic dysfunction.

Perspectives

355 The mechanisms underlying EICF are likely multifactorial, and importantly may differ between the RV
356 and LV. Previous research has proposed several influencing factors varying from β-adrenergic
357 receptor desensitization, oxidative stress, impaired calcium metabolism to altered post-exercise
358 loading. The influence of afterload conditions on RV EICF have rarely been explored. This study
359 demonstrated that, under hypoxic conditions at 3000m altitude (FiO_2 14.5%), the magnitude of EICF
360 is not augmented and thus it may be less likely that a role for elevated RV wall stress is relevant.
361 Although knowledge about the clinical long-term consequences of these temporary post-exercise
362 reductions in cardiac function is lacking, it has been hypothesized that this may be associated with
363 myocardial damage and worse clinical outcome. The absence of an effect in EICF between exercising
364 at sea level (normoxia) and 3000m altitude (hypoxia) is interesting, but long-term studies that link
365 these findings to prolonged follow-up is needed to better understand these findings. The novel
366 strain-area loop, introduced to assess haemodynamics non-invasively, provided substantial added
367 value in this study where it was sensitive enough to detect changes due to hypoxia. This novel
368 technique seems promising in providing physiological and pathophysiological insight and might be of
369 added value in clinical practice.[5, 27, 28, 37, 45-48]

370

371 **Limitations**

372 This study implemented a standardized exercise challenge to prevent a pre- and post-exercise
373 (para)sympathetic imbalance during echocardiographic evaluation. Instead of the methodology of
374 Stewart *et al.*[14] (aiming at 100 bpm), we set our target HR at 110-120 bpm during the exercise
375 challenge, to better mimic cardiac function during exercise. This higher HR may impede speckle
376 tracking quality. With current frame rates used, we experienced that tracking was still good to
377 excellent for LV global longitudinal strain and RV free wall strain. A further limitation is that we did
378 not obtain direct measures of RV wall stress as this would require invasive procedures. Alternatively,
379 we used only non-invasive echocardiographic, indirect measures to estimate any potential difference

in RV wall stress under hypoxia *versus* normoxia. When considering these indirect indices, some studies have demonstrated value of PAT during stress to estimate PAP in PAH patients whilst others have questioned the outside of the normal heart rate range (<60 or >100 bpm).[24, 26] It is clear that a more robust assessment of PAP would provide added support to the well-established physiological concepts and understanding of hypoxia and pulmonary haemodynamics. Previous studies have applied strain-area loops to PAH patients and demonstrated an association between PVR and the late diastolic uncoupling and the Sslope during rest only.[28] Further work should aim to validate the strain-area loops during stress. Finally, for technical reasons we only evaluated right heart function and haemodynamics during low-to-moderate stress echocardiography rather than during the high-intensity running exercise.

CONCLUSION

There was no impact of hypoxia on the magnitude of EICF in the RV and LV after a bout of 45-minute high-intensity exercise. This finding suggests that any potential increase in loading conditions does not automatically exacerbate EICF in this setting.

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527 **FIGURE LEGENDS**

528 **Figure 1**. Overview of study design, where the dotted panel is highlighting visit 2 and 3 (test days).

529

530 **Figure 2.** Schematic overview of the RV strain-area loop and the derived characteristics.

531 The black line represents the strain-area loop, the thick part represents the systolic phase and the thin line the
532 systolic phase. EDA, end-diastolic area. ESA, end-systolic area. ESslope, early systolic slope. Sslope, systolic
533 slope. Uncoupling ED, uncoupling end-diastolic. Uncoupling LD, uncoupling late diastolic.
534

535

536 **Figure 3**. Right ventricular longitudinal strain (A) and left ventricular longitudinal strain (B) prior to
537 and post 45-minutes high intensity running exercise. Error bars reflect the standard error of the
538 mean.

539 Linear mixed models factors:
540 E, Exercise: Comparison between all echocardiographic measurements performed pre *vs.* post 45-minutes high
541 intensity exercise.
542 H, Hypoxia: Comparison between all echocardiographic measurements performed under hypoxic *vs.* normoxic
543 conditions.
544 S, Stress: Comparison between all echocardiographic measurements performed during rest *vs.* during stress.
545 H*E, Hypoxia*Exercise: Comparison whether the change pre- *vs.* post-exercise (EICF) is different during hypoxic
546 *vs.* normoxic conditions.
547 E*S, Exercise*Stress: Comparison whether the change pre- *vs.* post-exercise is different measured during rest
548 *vs.* stress echocardiography.
549 E*H*S, Exercise*Hypoxia*Stress: comparison whether the change pre- *vs.* post-exercise under hypoxic *vs.*
550 normoxic conditions was different when observed using rest *vs.* stress echocardiography.
551

552 **Figure 4.** Right ventricular strain-area loops prior to and post 45-minute high intensity running
553 exercise during rest (A) and stress (B). Red and blue lines indicating normoxic and hypoxic exercise,
554 respectively. Solid and dotted lines reflecting pre- and post-exercise, respectively.

TABLES

Table 1. Subject characteristics

Sex (m/f)	14/7
Age (yr)	22.2±0.6
Height (cm)	170±2
Body Mass (kg)	70±2
BMI (kg/m2)	24.0±0.6
BSA (m2)	1.8±0.04
Resting HR (bpm)	65±2
Resting SBP (mmHg)	119±1
Resting DBP (mmHg)	69±2
Resting MAP (mmHg)	85±1
resting SpO2 (%)	98.4±0.3
VO_2max (L/min)	3.6±0.1
VO_2max/kg (mL/min/kg)	52±2
VE (L/min)	138±6
HRmax (bpm)	199±2

Data are expressed as means±SEM. m, male. f, female. BMI, body mass index. BSA, body surface area. HR, heart rate. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. SpO2, oxygen saturation. VO_2max, maximal oxygen uptake. VE, ventilation.

Table 2. Right ventricular function and mechanics during rest and stress pre- and post-exercise under normoxia and hypoxia

	Rest echocardiography				Stress echocardiography				p-values					
	Normoxia		Hypoxia		Normoxia		Hypoxia							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	H	E	S	H*E	E*S	E*H*S
Structure														
RV basal diameter (mm)	36.8±0.9	37.4±0.7	36.9±0.7	37.8±0.7	36.4±0.7	35.6±0.8	36.9±.7	37.1±0.6	0.13	0.45	**0.03**	0.25	0.10	0.36
RV mid-cavity diameter (cm)	28.9±0.8	29.4±0.6	29.4±0.9	29.9±0.7	28.8±0.7	28.2±0.8	28.9±.7	28.8±0.8	0.19	0.9	0.07	0.61	0.22	0.65
RVEDA (cm2)	20.4±0.7	20.3±0.6	20.7±0.7	21.4±0.7	19.9±0.6	19.7±0.8	20.3±0.6	20.3±0.7	**0.01**	0.56	**0.01**	0.14	0.38	0.46
RVESA (cm2)	10.8±0.5	11.2±0.4	11.0±0.4	11.8±0.4	9.8±0.3	10.3±0.5	9.9±0.4	10.8±0.5	0.14	**0.001**	**<0.001**	0.14	0.65	0.99
RVOTplax (mm)	24.0±0.7	24.4±0.8	25.5±0.6	25.6±0.5	24.2±0.7	23.0±0.7	24.6±0.7	24.1±0.7	0.07	0.22	**0.006**	0.55	**0.04**	0.21
RVOT1psax (mm)	24.8±0.8	25.6±0.7	26.1±0.7	25.9±0.6	25.2±0.6	24.2±0.6	25.9±0.9	25.1±0.7	0.15	0.34	0.15	0.36	0.08	0.11
RVOT2psax (mm)	16.6±0.4	16.7±0.4	17.3±0.4	16.8±0.4	17.0±0.5	16.6±0.5	17.1±0.4	17.0±0.5	0.17	0.28	0.62	0.75	0.82	0.21
RA area (cm2)	14.5±0.5	13.8±0.5	14.8±0.6	14.4±0.6	13.4±0.4	13.1±0.5	14.0±0.5	13.4±0.4	**0.04**	**0.001**	**0.001**	0.98	0.8	0.28
Function and mechanics														
RVFAC (%)	47±1	45±1	47±1	45±1	50±1	48±1	51±1	47±1	0.93	**0.007**	**<0.001**	0.49	0.43	0.36
TAPSE (cm)	27±1	26±1	28±1	26±1	30±1	28±1	30±1	28±1	0.89	**<0.001**	**<0.001**	0.52	0.20	0.9
TDI s' (cm/sec)	15±1	14±1	15±1	15±1	19±1	17±1	20±1	18±1	**0.02**	**0.002**	**<0.001**	0.55	0.11	0.73
TDI e' (cm/sec)	17±1	16±1	18±1	17±1	28±1	28±1	29±1	27±1	0.20	**0.02**	**<0.001**	0.45	0.61	0.45
TDI a' (cm/sec)	13±1	12±1	13±1	13±1	-	-	-	-	0.19	0.33	**-**	0.24	-	-
RV free wall strain (%)	-28.0±1	-27±1	-28±1	-28±1	-33±1	-30±1	-32±1	-30±1	0.90	**<0.001**	**<0.001**	0.58	**0.01**	0.86
RV time of peak (sec)	0.36±0.01	0.37±0.01	0.37±0.01	0.36±0.01	0.28±0.01	0.32±0.01	0.29±0.01	0.31±0.01	0.51	**<0.001**	**<0.001**	0.09	**0.004**	0.57
PAT (ms)	152±3	151±3	139±4	134±3	122±4	120±3	106±4	105±3	**<0.001**	**0.008**	**<0.001**	0.60	0.36	0.30
Strain-area loop characteristics														
Uncoupling (%)	2.0±0.2	1.0±0.4	1.4±0.3	0.6±0.4	1.4±0.5	1.2±0.5	0.7±0.4	0.3±0.5	0.07	0.05	0.32	0.99	0.23	0.66
Uncoupling ED (%)	2.0±0.3	1.0±0.4	1.4±0.3	0.6±0.4	1.4±0.5	1.3±0.5	0.7±0.5	0.4±0.6	0.10	0.10	0.40	0.92	0.14	0.7
Uncoupling LD (%)	2.0±0.2	1.1±0.3	1.3±0.3	0.6±0.4	1.5±0.4	1.0±0.5	0.8±0.4	0.1±0.5	**0.04**	**0.01**	0.22	0.96	0.67	0.62
Sslope (%/cm2)	2.5±0.1	2.5±0.1	2.4±0.1	2.4±0.1	2.8±0.1	2.7±0.1	2.6±0.1	2.6±0.1	0.07	0.53	**0.003**	0.8	0.35	0.33
Esslope (%/cm2)	2.4±0.2	2.6±0.1	2.4±0.1	2.6±0.1	2.9±0.2	2.7±0.2	2.9±0.2	2.7±0.2	0.85	0.97	**0.05**	0.87	**0.04**	0.88
Edslope (%/cm2)	1.4±0.1	1.9±0.2	1.7±0.1	1.8±0.2	1.8±0.2	1.8±0.2	1.7±0.2	2.2±0.3	0.41	0.08	0.29	0.73	0.82	**0.03**
Ldslope (%/cm2)	3.3±0.2	3.0±0.2	3.1±0.2	2.8±0.2	3.6±0.3	3.5±0.3	3.4±0.3	3.1±0.3	0.11	0.18	**0.02**	0.77	0.73	0.34

Data are expressed as means±SEM. ED, Early diastole. ES, Early systole. LD, Late Diastole. PAT, pulmonary acceleration time. PLAX, Parasternal long axis. PSAX, parasternal short axis. RA, Right atrium. RV, Right ventricle. RVFAC, RV fractional area change. RVEDA, Right ventricular end-diastolic area. RVESA, Right ventricular end-systolic area. RVOT, Right ventricular outflow tract. TAPSE, Tricuspid annular plane systolic excursion. TDI, Tissue Doppler imaging.

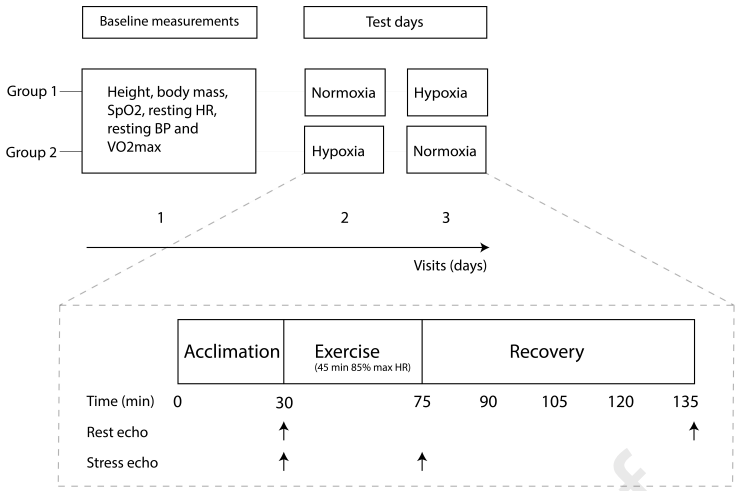
Linear mixed models factors:
H, Hypoxia: Comparison between all echocardiographic measurements performed under hypoxic *vs.* normoxic conditions.
E, Exercise: Comparison between all echocardiographic measurements performed pre *vs.* post 45-minutes high intensity exercise.
S, Stress: Comparison between all echocardiographic measurements performed during rest *vs.* during stress.
E*S, Exercise*Stress: Comparison whether the change pre- *vs.* post-exercise is different measured during rest *vs.* stress echocardiography.
H*E, Hypoxia*Exercise: Comparison whether the change pre- *vs.* post-exercise (EICF) is different during hypoxic *vs.* normoxic conditions.
E*H*S, Exercise*Hypoxia*Stress: comparison whether the change pre- *vs.* post-exercise under hypoxic *vs.* normoxic conditions was different when observed using rest *vs.* stress echocardiography.

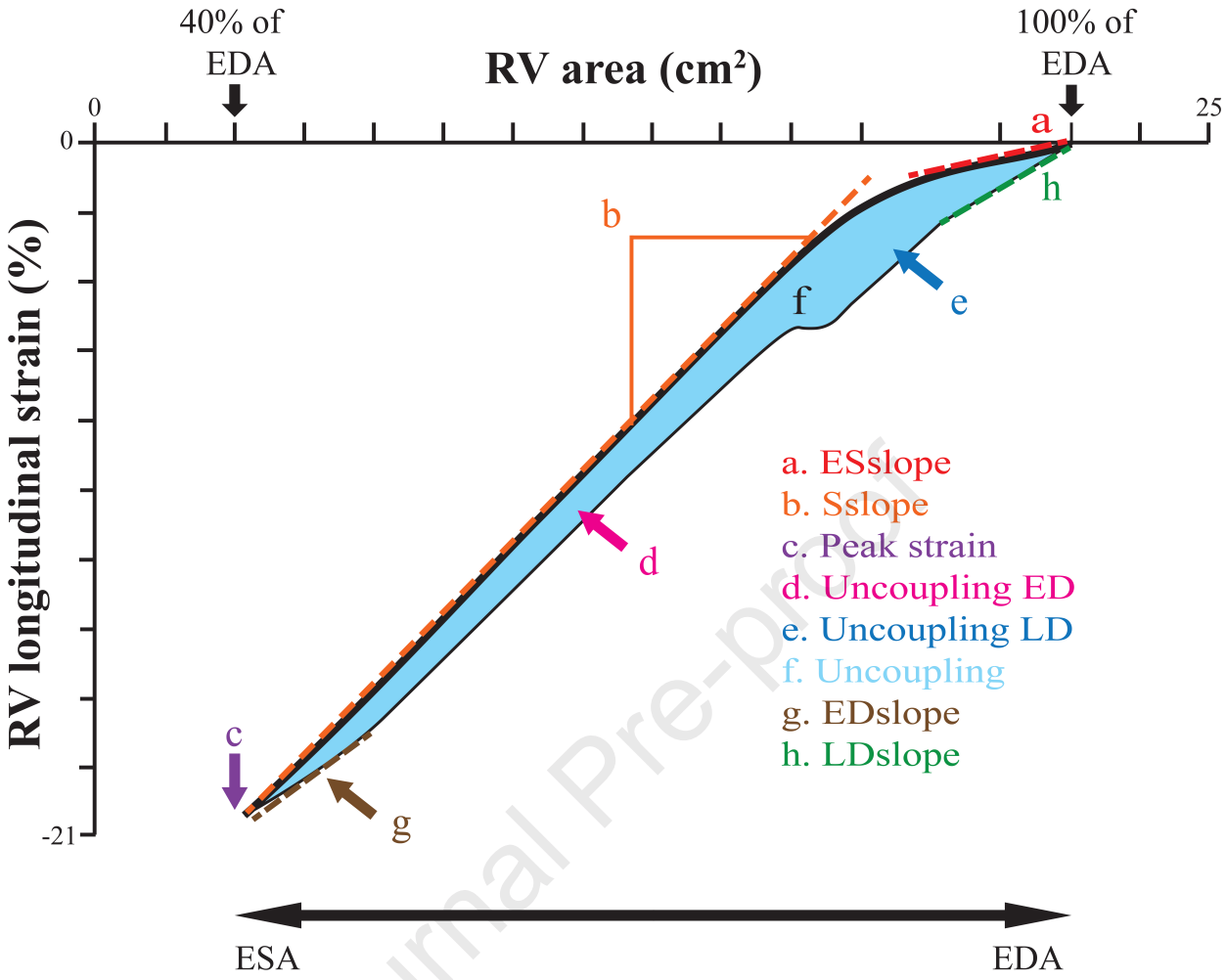
Table 3. Haemodynamics and left ventricular function and mechanics during rest and stress pre- and post-exercise under normoxia and hypoxia

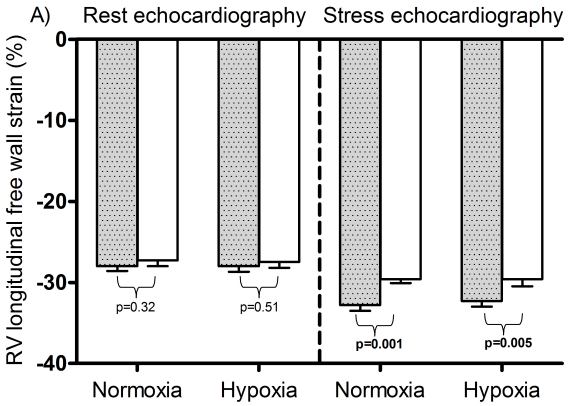
	Rest echocardiography				Stress echocardiography				p-values					
	Normoxia		Hypoxia		Normoxia		Hypoxia							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	H	E	S	H*E	E*S	E*H*S
Haemodynamics														
Heart rate (bpm)	68±2	71±3	74±2	79±3	113±1	112±1	111±1	113±1	**0.03**	0.07	**<0.001**	0.18	0.10	0.41
Systolic blood pressure (mmHg)	121±2	118±2	124±2	117±2	143±3	120±3	141±4	125±3	0.38	**<0.001**	**<0.001**	0.56	**<0.001**	**0.03**
Diastolic blood pressure (mmHg)	70±2	69±2	70±2	67±2	75±2	60±1	73±2	60±1	0.47	**<0.001**	0.16	0.98	**<0.001**	0.19
Mean arterial pressure (mmHg)	87±1	85±2	88±2	84±2	98±2	80±2	96±2	82±1	0.99	**<0.001**	0.08	0.75	**<0.001**	**0.04**
SpO2 (%)	98±0.2	98±0.3	90±0.5	90±0.7	94±0.8	95±0.6	82±1.0	83±0.9	**<0.001**	0.17	**<0.001**	0.36	0.86	0.68
Structure														
LVEDV (ml)	120±7	113±7	123±6	113±6	114±6	109±6	117±7	110±6	0.30	**<0.001**	**0.004**	0.46	0.55	1.0
LVESV (ml)	50±2	52±3	50±3	49±3	42±2	45±2	42±2	43±2	0.24	0.06	**<0.001**	0.17	0.12	0.76
LA diameter (mm)	30±1	29±1	30±1	28±1	31±1	27±1	30±1	27±1	0.79	**<0.001**	0.34	0.72	0.06	0.25
LA volume (ml)	38±2	34±0.3	39±2	34±2	38±1	35±1	39±1	36±1	0.21	**<0.001**	0.18	0.13	0.12	0.50
Function and mechanics														
LVEFbip (%)	58±1	56±1	59±1	56±1	63±1	58±1	65±1	60±1	**0.008**	**<0.001**	**<0.001**	0.69	**0.005**	0.44
TDI s' (cm/sec)	10±0.4	11±0.4	11±0.3	11±0.4	14±0.5	13±0.5	14±0.4	14±0.4	**0.04**	0.78	**<0.001**	0.98	0.24	0.19
TDI e' (cm/sec)	18±0.5	17±0.6	19±0.4	16±0.4	22±1.1	19±0.7	21±0.6	20±0.5	0.89	**<0.001**	**<0.001**	0.45	0.52	0.09
TDI a' (cm/sec)	8±0.4	9±0.3	9±0.3	10±0.3	-	-	-	-	**0.001**	0.054	-	0.60	-	-
E (cm/sec)	1.02±0.03	0.85±0.04	1.06±0.03	0.86±0.03	1.28±0.05	1.14±0.03	1.32±0.04	1.16±0.04	0.09	**<0.001**	**<0.001**	0.30	0.37	0.80
A (cm/sec)	0.56±0.02	0.57±0.02	0.55±0.02	0.62±0.03	-	-	-	-	**0.004**	0.39	-	0.18	-	-
E/A ratio	1.86±0.06	1.6±0.10	1.83±0.07	1.41±0.06	-	-	-	-	**0.048**	**<0.001**	-	0.15	-	-
LV longitudinal strain (%)	-20±0.3	-20±0.3	-21±0.3	-20±0.2	-23±0.3	-21±0.4	-24±0.4	-22±0.2	0.01	**<0.001**	**<0.001**	0.08	**0.05**	0.86
LV time of peak (sec)	0.36±0.01	0.36±0.01	0.35±.01	0.34±0.01	0.28±0.01	0.29±0.01	0.28±0.01	0.29±0.01	0.14	**0.03**	**<0.001**	0.22	**<0.001**	0.88

Data are expressed as means±SEM. ES, Early systole. LA, Left atrium. LVEDV, Left ventricular end-diastolic volume. LVESA, Left ventricular end-systolic volume. S, Stress. TDI, Tissue Doppler imaging.

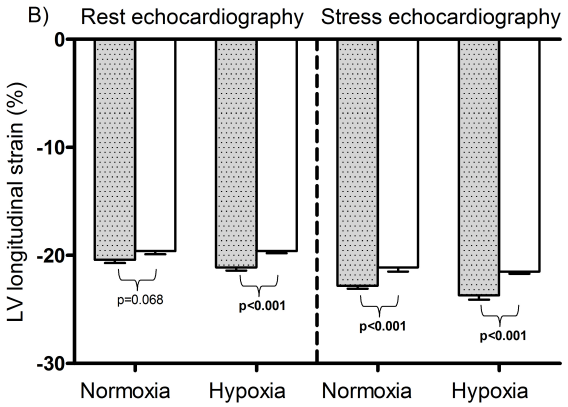
Linear mixed models factors:
H, Hypoxia: Comparison between all echocardiographic measurements performed under hypoxic *vs.* normoxic conditions.
E, Exercise: Comparison between all echocardiographic measurements performed pre *vs.* post 45-minutes high intensity exercise.
S, Stress: Comparison between all echocardiographic measurements performed during rest *vs.* during stress.
E*S, Exercise*Stress: Comparison whether the change pre- *vs.* post-exercise is different measured during rest *vs.* stress echocardiography.
H*E, Hypoxia*Exercise: Comparison whether the change pre- *vs.* post-exercise (EICF) is different during hypoxic *vs.* normoxic conditions.
E*H*S, Exercise*Hypoxia*Stress: comparison whether the change pre- *vs.* post-exercise under hypoxic *vs.* normoxic conditions was different when observed using rest *vs.* stress echocardiography.



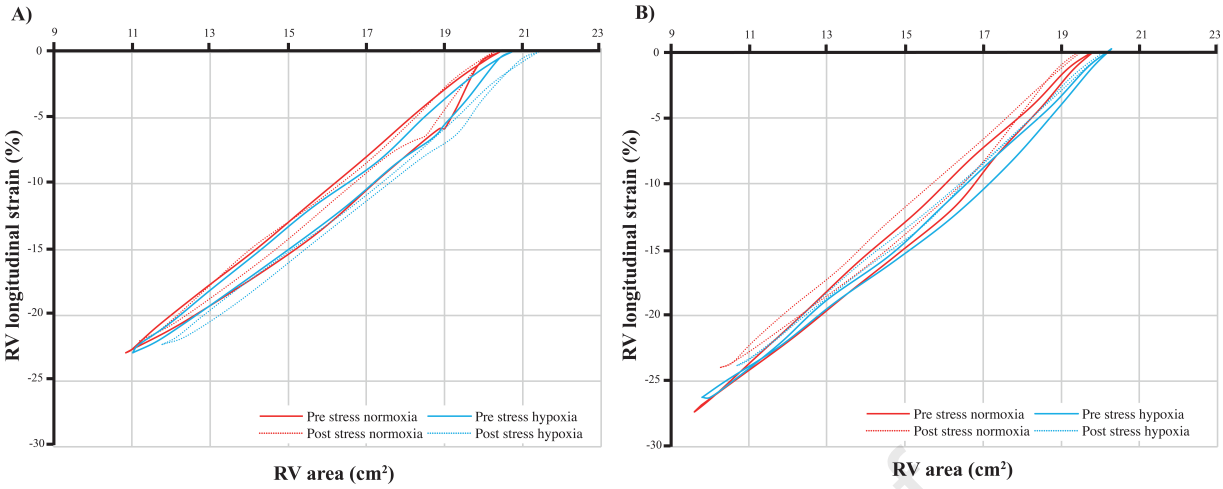




E: p<0.001; H: p=0.90; **S: p<0.001;**
H*E: 0.58; **E*S: 0.01;** E*H*S: p=0.86



E: p<0.001; H: p=0.01; **S: p<0.001;**
H*E: 0.08; **E*S: p=0.05**; E*H*S: p=0.86



HIGHLIGHTS

- 45-minutes high-intensity exercise induces right- and left-sided cardiac fatigue
- Exercise-induced cardiac fatigue is more pronounced during stress echocardiography
- Exercise under hypoxia does not exaggerate exercise-induced cardiac fatigue